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Can platelet-rich plasma be as effective as corticosteroids in the treatment of frozen shoulder in rats? Experimental animal study

Platelet-rich plasma and corticosteroids for frozen shoulder treatment

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Abstract

Aim: Frozen shoulder is an important health problem that causes significant socio-economic losses as it affects daily life. Its etiopathogenesis has not been fully elucidated. A long recovery period leads to new searches for treatment. In the treatment of frozen shoulder, corticosteroids (CS) and platelet-rich plasma (PRP) are applied, among other options. The aim of this study is to compare the effectiveness of CS and PRP in frozen shoulder.

Material and Methods: A secondary frozen shoulder model was created in rats. At the end of the eight-week waiting period, the shoulder joint was released. The rats, divided into three groups, were injected intraarticularly with saline in the first group, CS in the second group, and PRP in the third group. After a two-week waiting period, the shoulder joint range of motion of the sacrificed rats was measured. The joint capsule was evaluated histologically for synovial inflammation, type 3 collagen, capillary proliferation, subscapular bursa adhesion and fibrosis.

Result: CS and PRP application did not affect the range of motion of the joint. A significant increase in synovial inflammation a decrease in fibrosis, type 3 collagen deposition, subscapular bursa adhesion were revealed. It was observed that vascular proliferation did not change.

Discussion: CS and PRP treatment yielded similar results in the frozen shoulder animal model. While both of these treatments do not affect the range of motion of the joint, they reverse the pathological changes in a frozen shoulder.

Keywords

Frozen Shoulder, Corticosteroid, Platelet-Rich Plasma, Joint Range of Motion

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Introduction

Frozen shoulder (FS), with pain and limitation in joint range of motion, is a common disorder of the glenohumeral joint, but its etiopathogenesis has not been fully elucidated. Complaints usually regress on their own in 1-3 years, but in some cases, this period has been reported to last up to 10 years [1]. It is mainly seen in individuals between the ages of 40 and 60 who are active and constitute a productive power. Considering the prolongation of the recovery period of up to 3-10 years in this disease, which severely restricts daily life, it causes significant socio-economic losses and psychological problems for both the patient and the society [1].

The pathophysiology of FS is not fully understood, but several mechanisms have been suggested. Inflammatory changes, fibrosis and capsular contracture are part of the pathological changes [2]. The pathophysiology of FS is firstly the pathological changes caused by synovial inflammation followed by the development of capsular fibrosis. But the cause of FS is still unknown. Treatment modalities for FS include drug therapy, local steroid injection, physiotherapy, hydrodistention, manipulation under anesthesia, arthroscopic capsule release, and open capsule release [3].

It has been reported that CS treatment in FS is beneficial both in relieving pain and increasing joint range of motion (JRM) [4]. PRP is a fraction of whole blood containing concentrated growth factors and proteins. Cytokines contained in PRP drive tissue healing through autocrine and paracrine effects [5]. It has been reported that PRP injections can be used as a safe and non-surgical intervention to reduce FS pain and improve shoulder mobility [6].

The aim of this study is to determine the effectiveness of CS and PRP treatments in FS and to compare the difference between them.

Material and Methods

Ethical approval was obtained for this study with the decision of Başkent University Medical and Health Sciences Research Board and Experimental Animals Ethics Committee dated 30/01/2017 and numbered 17/02.

In our study, 48 Sprague Dawley, 8-month-old female rats were used. The weight of the rats used ranged from 250 to 300 g. Before being included in the study, subjects were verified to have no gait disorders. Each animal was housed in polycarbonate cages of 8 rats in an environment where they could freely get food and water. Ambient humidity was constant at 50±10% and ambient temperature was 20±2 °C as a standard. The room in which the cages were located was illuminated for 12 hours, from eight in the morning to eight in the evening, in a day-night cycle. During the experiment, the rats were given Purina® standard rat chow. The animals were not treated with activity restriction or rehabilitation (treadmill) throughout the experiment.

The subjects were divided into three groups of sixteen. Half of the subjects in each group were used for JRM measurement (n=8) and the other half for histopathological examination (n=8).

Blood was taken from the tail vein of Group 3 subjects into 1 ml citrate tubes. After centrifugation at 700 RCF for 7 minutes, the plasma at the top of the tube was taken with an injector and prepared for intraarticular injection from the posterior left shoulder of the same rat.

All animals underwent the same standard surgical operation by the same surgeon. Preoperatively, 50mg/kg Ketamine Hydrochloride (Brema®, Bremer Pharma GMBH, Warburg - Germany) and 7 mg/kg Xylazine Hydrochloride (Alfazyne®, Alfasan International B.V., Woersen – Netherlands) were administered intraperitoneally for general anesthesia. The subjects were numbered by writing numbers on their tails. After anesthesia, the rats were weighed and the weights were noted. The left shoulder surgical field was free of hair. The subject was fixed by taping the right front and both hind legs onto the styrofoam in the prone position. The surgical site was cleaned with 10% polyvinylpyrrolidone – iodine-containing antiseptic solution (Batticon®) and covered with a sterile drape. All surgical procedures were performed under sterile conditions.

FS in rats was created in all three groups by modeling the previous study by Villa-Camacho et al. [7]. After this procedure, 10mg/kg of enrofloxacin (Baytril-K®) was given subcutaneously for three days as an antibiotic, but no anti-inflammatory drug was used. At the end of eight weeks of waiting, the sutures were cut and the shoulder was released. Intraoperatively, the range of motion of the joint was examined passively without forcing the shoulder, and the release of the sutures was confirmed.

Group 1 (Control): 1 ml of 0.9% saline solution (SF) to the left shoulder joints of rats. Group 2 (CS): 0.2 ml, single dose 0.5 mg/kg triamcinolone acetonide (Kenakort®-A retard IM/IA 40 mg ampoule, Deva İlaç, Istanbul-Turkey). Group 3 (PRP): In the same session, from the tail vein of rats. PRP obtained from the blood taken was applied intra-articularly from the posterior joint.

In the tenth week (two weeks after injection), all groups were sacrificed by administering euthanasia dose of intraperitoneal Ketamine Hydrochloride (150mg/kg). The treated left shoulders of the subjects were removed en bloc to include the forearm, humerus, clavicle and scapula. A randomly selected half of the sacrificed animals from all three groups (n=8, each group) were reserved for JRM measurement and the other half (n=8, each group) for histopathological examination.

All measurements were performed in the same way in the standard setup. For the experiment, a horizontal line was drawn on the Styrofoam, parallel to the bottom edge of the Styrofoam. Angle values were marked on the lower and upper parts of the line and divided into 2 parts as adduction (Add) and abduction (Abd).

A standard weight of 10 grams (gr) was used for all measurements. The measurements were made before the shoulder joints began to degenerate after sacrification. The tissues were kept moist by dripping saline during the procedures. JRM measurement was performed as previously described by Oki et al. [8].

Tissue samples taken by making coronal sections (parallel to the scapula) from the shoulder joints were subjected to routine histopathological procedures. It was stained with Hematoxylin-Eosin (H&E) stain to evaluate synovial inflammation, type 3 collagen, capillary proliferation, and subscapular bursa adhesion. Picro Sirius Red was stained to evaluate fibrosis. It was evaluated under the light microscope at appropriate

magnification.

Statistical Analysis

SPSS 22.0 package program was used for statistical analysis of the data. Categorical measurements were presented as numbers and continuous measurements as Mean±Standard Deviation (SD). The normal distribution of the groups was examined using the Shapiro–Wilk test. Tukey's multiple comparison test was used for JRM analysis. Fisher's test was used to analyze histological studies.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

When the groups were compared with each other for joint range of motion, no significant difference was found between them (Table 1 and Figure 1).

Compared to the control group, synovial inflammation was significantly increased in the CS and PRP group (x^2=7.273; p=0.007). There was no significant difference between the CS and PRP groups. When the groups were compared among themselves in terms of vascular proliferation, no significant difference was found (x^2=1.067; p= 0.3017). Fibrosis was found to be significantly reduced in the CS and PRP groups when compared to the control group (x^2=5.923; p= 0.0149). Compared to the control group, Type 3 collagen deposition was found to be significantly reduced in the CS (x^2=12.44; p=0.0004) and PRP (x^2=7.273; p=0.007) groups. Subscapular bursa adhesion was found to be significantly reduced in the CS (x^2=5.333; p=0.02) and PRP (x^2=5.333; p=0.02) groups compared to the control group (Table 1).

Table 1. The effect of CS and PRP treatment on JRM and histological parameters in rats with frozen shoulder model.

		Control (n=8)	CS (n=8)	PRP (n=8)	SM
Joint Range of Motion	Add	32.17±9.4	40.0±10.4	38.0±5.2	*
	Abd	38.13±9.2	42.25±7.1	37.25±8.4	*
	Total	70.75±11.4	82.13±10.8	77.63±12.0	*
Synovial Inflammation (n)	No	3	8	8	**
	Mild	5	0	0	
	Severe	0	0	0	
Vascular proliferation (n)	No	6	6	4	**
	Yes	2	2	4	
Fibrosis (n)	No	1	7	7	**
	Mild	4	1	1	
	Severe	3	0	0	
Type 3 Collagen Deposition (n)	No	0	7	5	**
	Yes	8	1	3	
Subscapular Bursa Adhesion (n)	No	4	8	8	**
	Yes	4	0	0	

Add: Adduction; Abd: Abduction; SM: statistical method *Tukey's multiple comparison test; **Fisher's test

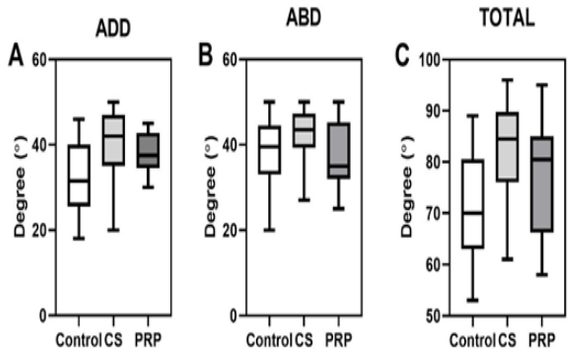

Figure 1. The effect of corticosteroid and PRP treatment on joint range of motion (Add: Adduction; Abd: Abduction).

Discussion

In this study, it was observed that the effect of CS and PRP treatment on JRM was limited in rats with the FS model. On the other hand, it was found that synovial inflammation increased significantly, fibrosis, type 3 collagen deposition, and subscapular bursa adhesion decreased. It was observed that vascular proliferation did not change. CS and PRP act similarly in the treatment of FS.

Intra-articular CS injection is one of the FS treatment protocols [1]. It has been reported that intraarticular CS leads to rapid relief of pain and improvement of JRM [1, 9, 10]. However, in this study, it was seen that CS treatment did not change JRM. On the other hand, parameters such as synovial inflammation, fibrosis, type 3 collagen deposition and subscapular bursa adhesion, which play a role in the pathophysiology of FS, were found to have changed positively. It has been suggested that the increase in fibroblasts in the joint capsule in FS plays a role in the regulation of inflammatory and fibrotic processes [11]. Capsular fibrosis and contracture have been suggested to stiffen the shoulder capsule, limiting the range of motion [12]. In this study, it was found that synovial inflammation increased, whereas fibrosis decreased. This situation was interpreted to mean that both CS and PRP had a therapeutic effect.

It has been shown that the amount of type 3 collagen in the capsule and the adhesion to the subscapular bursa increase in the rat FS model [13]. It has been reported that the synovial fluids of FS patients have high collagen synthesis [14]. In this study, it was found that the amount of type 3 collagen decreased as a result of CS and PRP treatment. The increase in fibroblasts in the synovial fluid increases the amount of type 3 collagen. We think that the decrease in the amount of type 3 collagen shows the therapeutic effect of both CS and PRP. On the other hand, the decrease in subscapular bursa adhesion as a result of both CS and PRP treatments shows the effectiveness of both treatment methods.

Thu et al. reported that adhesive capsulitis intra-articular PRP injection may be a useful option in the treatment of patients with low therapeutic compliance, especially for exercise therapy or contraindications for CS injection or oral pain-reducing drugs [15]. In a study conducted by Feusi et al. on rats, they reported that PRP injections corrected the histological changes in the frozen shoulder [16]. The findings obtained in this study support the findings of Feusi et al. However, we found that both CS and PRP treatment did not affect JRM. According to the histological results, CS and PRP, whose therapeutic effects were also shown in this study, were not effective on JRM; It has been interpreted that physical therapy-assisted CS and PRP application could increase JRM. On the other hand, the reason why CS and PRP treatment did not make a significant difference in JRM in this study may be due to the use of a 10 g weight to measure JRM. In

pilot studies, 10-13% of humeral shaft fractures were observed when 20 and 30 grams of weight were used; We evaluated the JRM using a 10- gram weight for measurements.

It is reasonable to investigate FS in an animal model to better define the etiology, pathology, and treatment, which are not fully clear. Since it is not possible to create primary FS on an animal model, it is preferred to create a secondary FS model with joint immobilization in studies. Immobilization is the second most common cause of secondary frozen shoulder after diabetes. Although it is advantageous to perform surgery on large animals such as rabbits and dogs, rats are preferred in many studies because they are easy to obtain, have more anatomical similarities to the human shoulder and are more resistant to surgical procedures. Researchers reported that the shoulder movements of rats during forward walking were similar to the abduction movements of humans [17]. The most obvious limitation of JRM in FS is in the direction of external rotation in humans. On the other hand, it is known to be in the abducent direction in the rat model [18, 19]. In our study, the improvement in the abducent direction was higher in the groups in which recovery was investigated, which is an expected result since this is the direction that rats use most in shoulder movements during walking.

Limitation

The limitation of this study is that all subjects were left in their natural course without any coercion similar to rehabilitation. In this study, there was no difference between the treatment groups and the control group in terms of JRM. We think that the JRM will increase if the subjects are forced similar to rehabilitation.

Conclusion

As a result, there is no difference between CS and PRP application in the treatment of FS, these treatments do not affect JRM, but the levels of molecules that play a role in the pathophysiology of FS have a therapeutic effect. It is thought that the use of CS and PRP application together with other options such as physical therapy in FS may increase JRM.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

The authors declare no conflict of interest.

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